

Claims

1. A method of producing pluripotent stem cells, which comprises culturing testis cells using a medium containing
5 glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells.
2. The production method of claim 1, wherein the medium further contains leukemia inhibitory factor (LIF).
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3. The production method of claim 1 or 2, wherein the medium further contains at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).
- 15 4. The production method of any one of claims 1 to 3, which comprises culturing testis cells in the presence of feeder cells.
5. The production method of claim 1, wherein the testis cells
20 are spermatogonial stem cells.
6. The production method of claim 5, wherein the spermatogonial stem cells are GS cells.
- 25 7. The production method of claim 1, wherein the testis cells are P53-deficient.
8. The production method of claim 1, which comprises the following steps:
30 (Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain cultured cells;
(Step 2) culturing the cultured cells obtained in Step 1, using a medium containing leukemia inhibitory factor (LIF) to obtain
35 pluripotent stem cells.

9. The production method of claim 8, wherein the medium for Step 1 further contains leukemia inhibitory factor (LIF).
- 5 10. The production method of claim 8 or 9, wherein the medium for Step 1 further contains at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).
11. The production method of any one of claims 8 to 10, wherein
10 Step 1 comprises culturing testis cells in the presence of feeder cells.
12. The production method of claim 1, which comprises the following steps:
 - 15 (Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain GS cells;
 - (Step 2) culturing the GS cells obtained in Step 1, using a medium containing glial cell derived neurotrophic factor (GDNF)
 - 20 or an equivalent thereto to obtain pluripotent stem cells.
13. The production method of any one of claims 1 to 12, wherein the testis cells are derived from a mammal.
- 25 14. The production method of claim 13, wherein the mammal is postnatal.
15. The production method of claim 1, wherein the pluripotent stem cells are positive for at least any one selected from the
30 group consisting of SSEA-1, Forsman antigen, β 1-integrin, α 6-integrin, EpCAM, CD9, EE2 and c-kit.
16. The production method of claim 15, wherein the pluripotent stem cells are positive for SSEA-1, Forsman antigen, β 1-integrin, α 6-integrin, EpCAM, CD9, EE2 and c-kit.

17. A pluripotent stem cell produced by the production method of any one of claims 1 to 16.

5 18. A pluripotent stem cell derived from a testis cell, which is positive for at least any one selected from the group consisting of SSEA-1, Forsman antigen, β 1-integrin, α 6-integrin, EpCAM, CD9, EE2 and c-kit.

10 19. The pluripotent stem cell of claim 18, which is positive for SSEA-1, Forsman antigen, β 1-integrin, α 6-integrin, EpCAM, CD9, EE2 and c-kit.

15 20. A method of producing a chimeric embryo, which comprises the following steps:
(Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;
(Step 2) introducing the pluripotent stem cells into a host
20 embryo to obtain a chimeric embryo.

21. A method of producing a chimeric animal (excluding humans), which comprises the following steps:
(Step 1) culturing testis cells using a medium containing glial
25 cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;
(Step 2) introducing the pluripotent stem cells into a host embryo to obtain a chimeric embryo;
(Step 3) transferring the chimeric embryo to the uterus or
30 oviduct of a host animal to obtain a chimeric animal (excluding humans).

22. A method of producing a non-human animal derived from pluripotent stem cells, which comprises the following steps:
35 (Step 1) culturing testis cells using a medium containing glial

cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;

(Step 2) introducing the pluripotent stem cells into a host embryo to obtain a chimeric embryo;

5 (Step 3) transferring the chimeric embryo to the uterus of a host animal to obtain a chimeric animal (excluding humans);
(Step 4) mating the chimeric animal to obtain a non-human animal derived from the pluripotent stem cells.

10 23. A method of producing a tetraploid chimeric embryo, which comprises the following steps:

(Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;

15 (Step 2) introducing the pluripotent stem cells into a tetraploid embryo to obtain a tetraploid chimeric embryo.

24. A method of producing a non-human animal derived from pluripotent stem cells, which comprises the following steps:

20 (Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;

(Step 2) introducing the pluripotent stem cell into a tetraploid embryo to obtain a tetraploid chimeric embryo;

25 (Step 3) transferring the tetraploid chimeric embryo to the uterus or oviduct of a host animal to obtain a non-human animal derived from the pluripotent stem cells.

30 25. A method of producing functional cells, which comprises the following steps:

(Step 1) culturing testis cells using a medium containing glial cell derived neurotrophic factor (GDNF) or an equivalent thereto to obtain pluripotent stem cells;

35 (Step 2) culturing the pluripotent stem cells under functional cell differentiation conditions to obtain functional cells.

26. The production method of claim 25, wherein the functional cells are mesodermal cells.

⁵ 27. The production method of claim 26, wherein the mesodermal cells are any one selected from the group consisting of blood cell lineage cells, vascular lineage cells and myocardial cells.

¹⁰ 28. The production method of claim 25, wherein the functional cells are ectodermal cells.

29. The production method of claim 28, wherein the ectodermal cells are neuronal lineage cells.

¹⁵ 30. The method of claim 29, wherein the neuronal lineage cells are any one selected from the group consisting of neurons, glial cells, oligodendrocytes and astrocytes.

²⁰ 31. The production method of claim 25, wherein the functional cells are endodermal cells.

32. A composition for producing pluripotent stem cells derived from a testis cell, which contains glial cell derived ²⁵ neurotrophic factor (GDNF) or an equivalent thereto.

33. The composition of claim 32, which further contains leukemia inhibitory factor (LIF).

³⁰ 34. The composition of claim 32 or 33, which further contains at least one of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).